[ SEQ. ID NO: 3] X-C-C-T-T-G-A-G-A-T-T-T-C-C-C-T-C 5'

G-G-A-A-C-T-C-T-A-A-A-G-G-G-A-G-X
[ SEQ. ID NO: 4]



X-C-C-T-T-G-A-G-A-T-T-T-C-C-C-T-C G-G-A-A-C-T-C-T-A-A-G-G-G-A-G-X

FIG.2

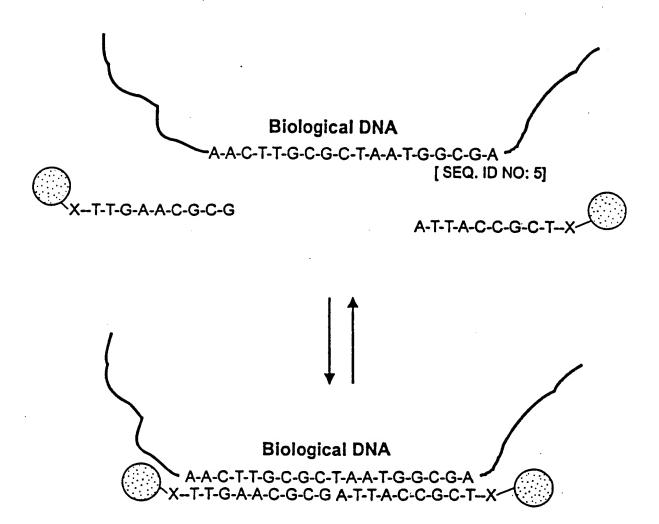
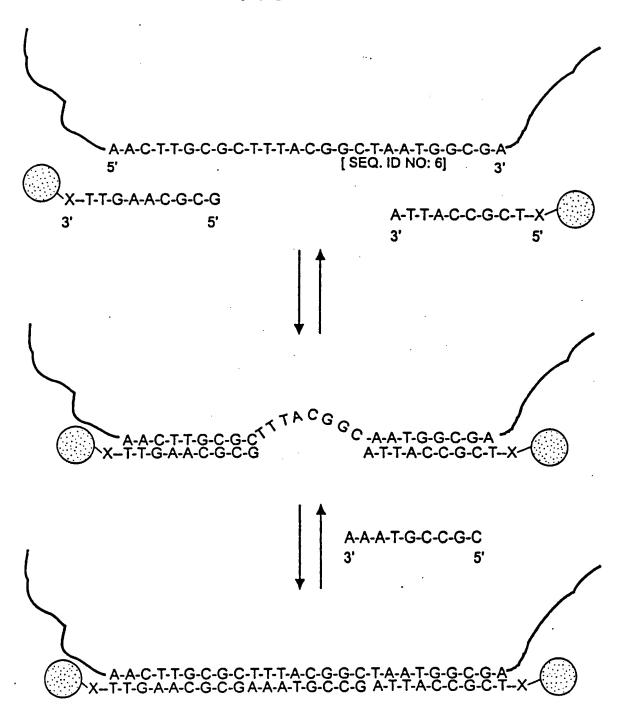


FIG.3



Linking oligonucleotide 5.
A-T-G-G-C-A-A-C-T-A-T-A-C-G-C-G-C-T-A-G
A-T-G-C-G-C-G-C-T-C-T-C-A-G-C-A-A-A
in NO: 21 3' 3' 1' 5' 5' [ SEQ. ID NO: 2]

.

Colloids

X-T-A-C-C-G-T-T-G

A-G-T-C-G-T-T-X-

Heat Mix below Tm

3' 5' X-T-G-G-C-A-A-C-T-A-T-A-C-G-C-G-C-T-A-G A-G-T-C-G-T-T-T-X X-T-A-C-C-G-T-T-G A-T-A-T-G-C-G-C-G-A-T-C-T-C-A-G-C-A-A-A 3' 5' Stand below Tm Heat

Aggregate

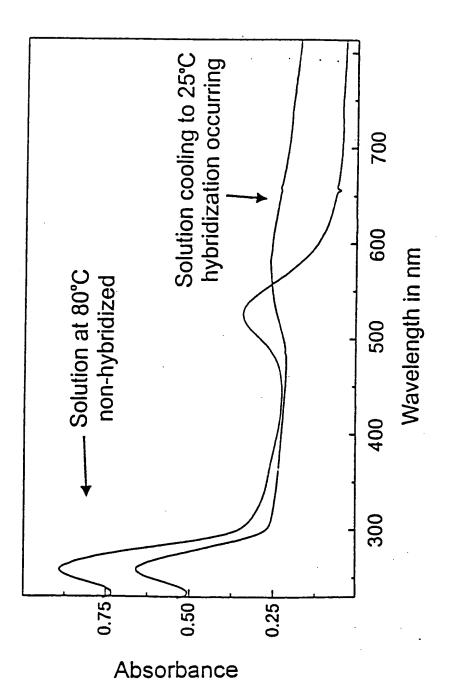
Precipitate (formed by further cross-linking)

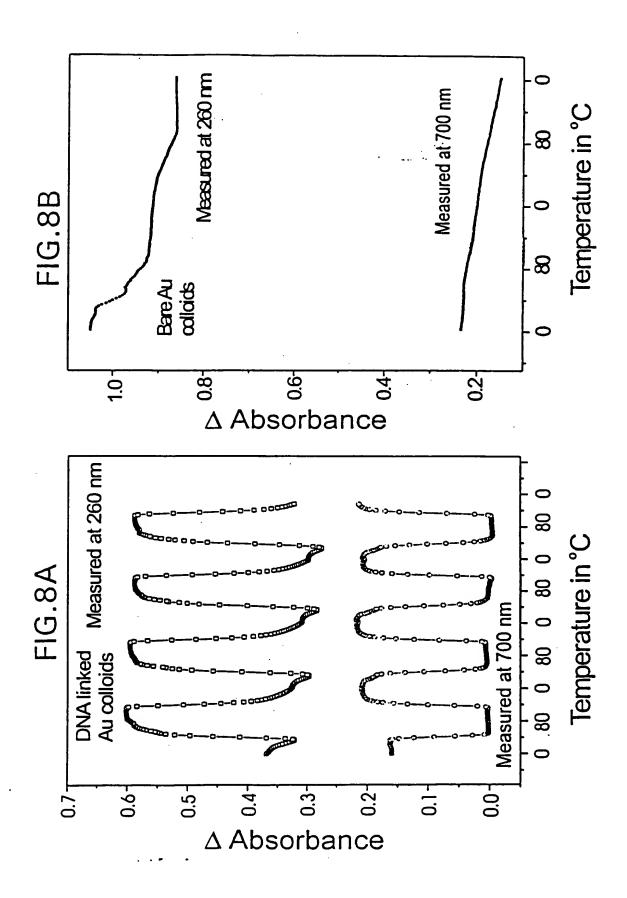
FIG.5 Au nanoparticles Modification with Modification with 3' thiol TACCGTTG 5' 5' AGTCGTTT 3' thiol Addition of linking DNA duplex 5'ATGGCAAC TITTTTCAGCAAA 5' Further oligomerization and settling



FIG.6A FIG.6B FIG.6C

FIG. 7





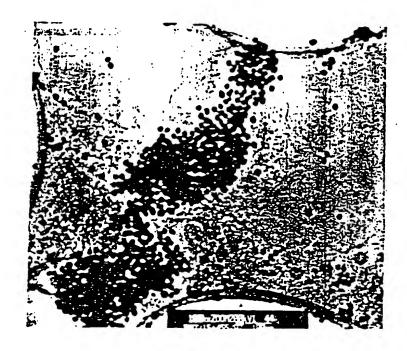


FIG.9A

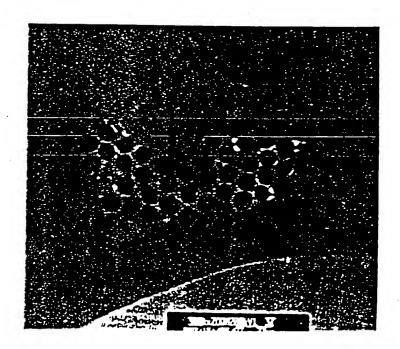
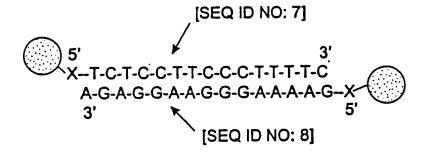
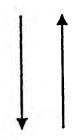


FIG.9B



3' T-C-T-C-C-T-T-C-C-C-T-T-T-T-C 5' [SEQ ID NO: 9]



5' 3' X--T-C-T-C-C-T-T-C-C-C-T-T-T-C A-G-A-G-G-A-A-G-X T-C-T-C-C-T-T-C-C-C-T-T-T-C 5'

[SEQ. ID NO: 10]

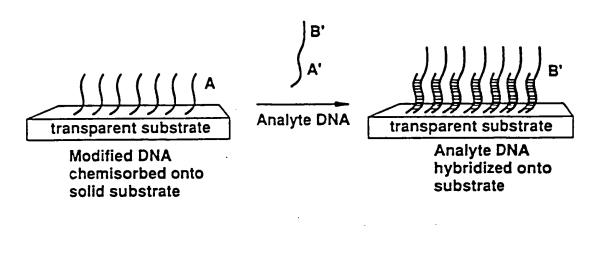
5' S-A-T-G-G-C-A-A-C-T-A-T-Ā-C-G-C-G-C-T-A-G-A-G-T-C-G-T-T-T-3'

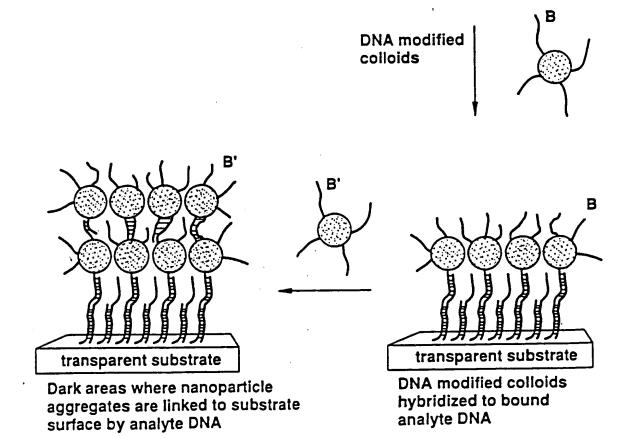
\[SEQ. ID NO: 11] T-A-C-C-G-T-T-G-A-T-A-T-G-C-G-C-G-A-T-C-T-C-A-G-C-A-A-A-S-3'



FIG.12A Complementary Target [SEQ. ID NO: 14] [SEQ. ID NO:12] 3' T-C-G-T-A-C-C-A-G-C-T-A-T-C-C T-T-T-G-C-T-G-A-G-A-T-C-G-C-G 5' A-G-C-A-T-G-G-T-C-G-A-T-A-G-G-A-A-A-C-G-A-C-T-C-T-A-G-C-G-C FIG.12B [SEQ. ID NO:13] **Probes without Target** 3' T-C-G-T-A-C-C-A-G-C-T-A-T-C-C T-T-T-G-C-T-G-A-G-A-T-C-G-C-G FIG.12C Half Complementary Target 3' T-C-G-T-A-C-C-A-G-C-T-A-T-C-C T-T-T-G-C-T-G-A-G-A-T-C-G-C-G 5' A-G-C-A-T-G-G-T-C-G-A-T-A-G-G-A<del>[T-G-G-C]</del>A<del>[A-C-T-A-T-A]</del>C-G-C [SEQ. ID NO: 15] FIG.12D Target - 6 bp 3' T-C-G-T-A-C-C-A-G-C-T-A-T-C-C T-T-T-G-C-T-G-A-G-A-T-C-G-C-G 5' G-T-C-G-A-T-A-G-G-A-A-A-C-G-A-C-T-C-T-A-G-C-G-C [SEQ. ID NO:16] FIG.12E One bp Mismatch 3' T-C-G-T-A-C-C-A-G-C-T-A-T-C-C T-T-T-G-C-T-G-A-G-A-T-C-G-C-G 5' A-G-C-A-T-G-G-T-TG-A-T-A-G-G-A-A-A-C-G-A-C-T-C-T-A-G-C-G-C [SEQ. ID NO: 17] FIG.12F Two bp Mismatch 3' T-C-G-T-A-C-C-A-G-C-T-A-T-C-C T-T-T-G-C-T-G-A-G-A-T-C-G-C-G 5' A-G-C-A-T-GTTTG-A-T-A-G-G-A-A-A-C-G-A-C-T-C-T-A-G-C-G-C [SEQ. ID NO: 18]

## FIG.13A





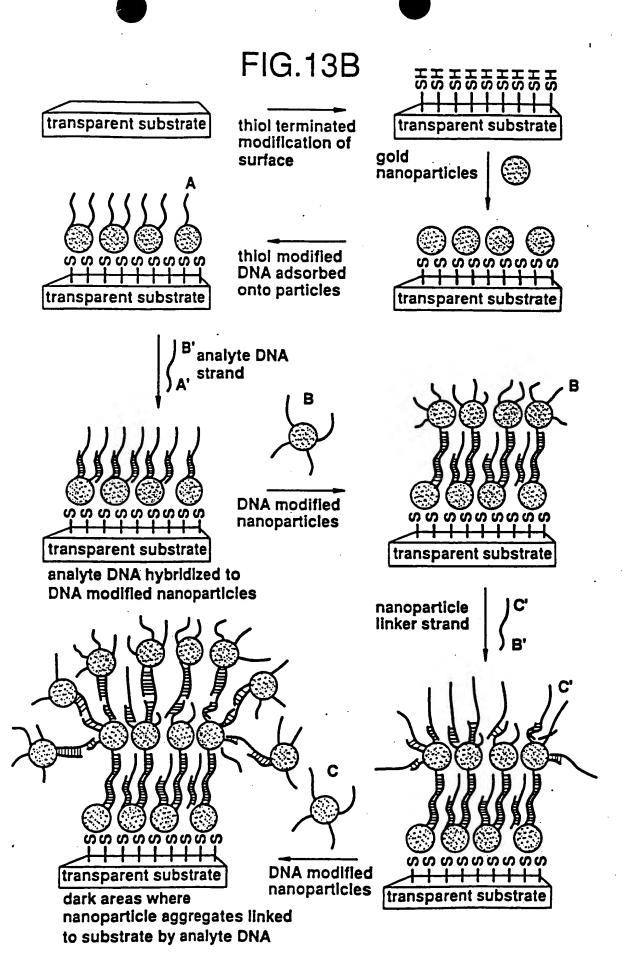


FIG.14A 0.35 2nd layer of Au nanoparticles: attached through DNA 0.25 Absorbance 0.15 Au nanoparticles: attached to glass substrate 0.05 700 800 600 400 500 Wavelength in nm FIG.14B 0.34 Absorbance at 520 nm First Derivative: Tm=62 C 0.30 0.26 0.22 40 60 80 20 40 60 Temperature in °C 80 20 60

:
FIG15A
Probes with No Target SEQ ID NO:19
SEQ ID NO:20
S-ATG-CTC-AAC-TCT TAG-GAC-TTA-CGC-S
FIG15B
Half-Complementary Target
SEQ ID NO:21
5' TAC-GAG-TTG-AGA-GAG-TGC-CCA-CAT 3'
S-ATG-CTC-AAC-TCT TAG-GAC-TTA-CGC-S
FIG15C 1 2
Complementary Target Tm=53.5°C
4 SEQ ID NO:22
5' TAC-GAG-TTG-AGA-ATC-CTG-AAT-GCG 3'
S-ATG-CTC-AAC-TCT TAG-GAC-TTA-CGC-S  1 2
FIG15D
ONE Base-Pair Mismatch at Probe Head Tm=50.4°C
5 SEQ ID NO:23
5' TAC-GAG-TTG-AGA-ATC-CTG-AAT-GC <u>T</u> 3' S-ATG-CTC-AAC-TCT TAG-GAC-TTA-CGC-S
1 2
FIG15E Tm=46.2°C
ONE Base-Pair Mismatch at Probe Tail
6 SEQ ID NO:24
5' TAC-GAG-TTG-AGA-CTC-CTG-AAT-GCG 3'
S-ATG-CTC-AAC-TCT TAG-GAC-TTA-CGC-S  1 2
FIG15F ONE Base Deletion  Tm=51.6°C
5' TAC-GAG-TTG-AGA-ATC-CTG-AAT-GC 3'
S-ATG-CTC-AAC-TCT TAG-GAC-TTA-CGC-S  1 2
FIG15G
ONE Base-Pair Insertion Tm=50.2°C
SEQ ID NO:26
5' TAC-GAG-TTG-AGA-CAT-CCT-GAA-TGC-G 3'
S-ATG-CTC-AAC-TCT TA-GGA-CTT-ACG-C-S

## 24 Base Template FIG. 16A

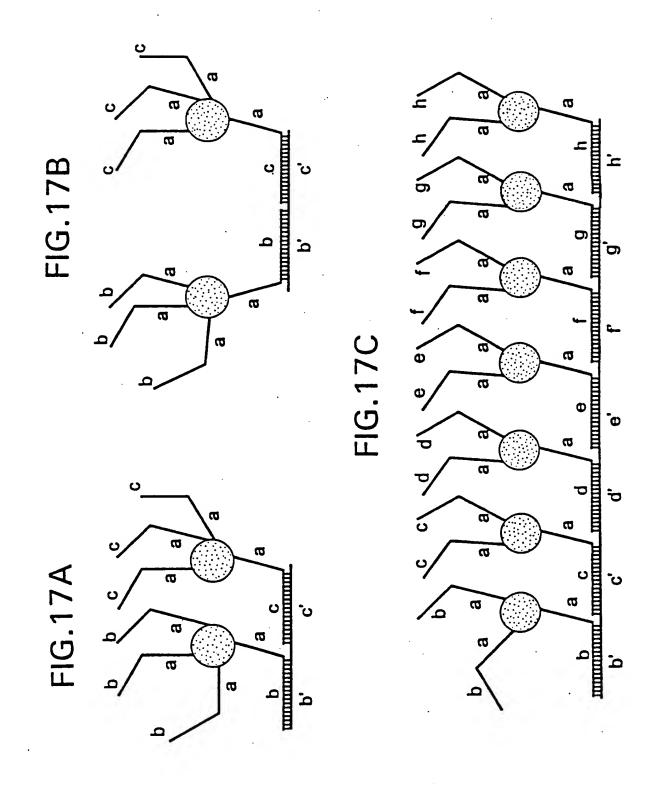
- S-ATG-CTC-AAC-TCT TAG-GAC-TTA-CGC-S 5' TAC-GAG-TTG-AGA-ATC-CTG-AAT-GCG 3'

## 48 Base Template with Complementary 24 Base Filler FIG. 16B

~ S-ATG-CTC-AAC-TCT GGC-AAT-TCT-GCT-CCG-TTA-GTA-CGT TAG-GAC-TTA-CGC-S 5' TAC-GAG-TTG-AGA-CCG-TTA-AGA-CGA-GGC-AAT-CAT-GCA-ATC-CTG-AAT-GCG 3'

# 72 Base Template with Complementary 48 Base Filler FIG.16C

5' TAC-GAG-TTG-AGA-CCG-TTA-AGA-CGA-GGC-AAT-CAT-GCA-TAT-ATT-GGA-CGC-TTT-ACG-GAC-AAC-ATC-CTG-AAT-GCG 3'



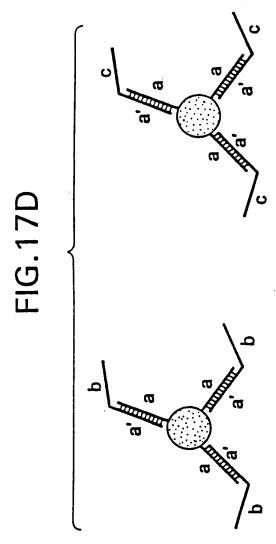
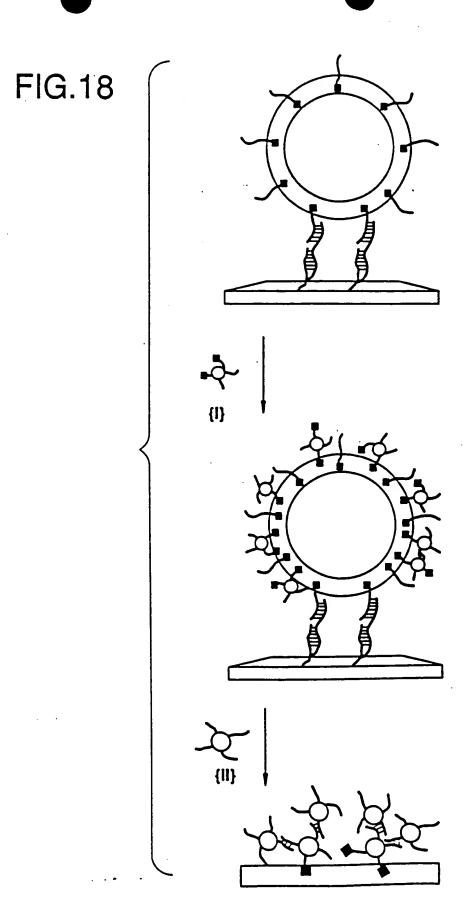
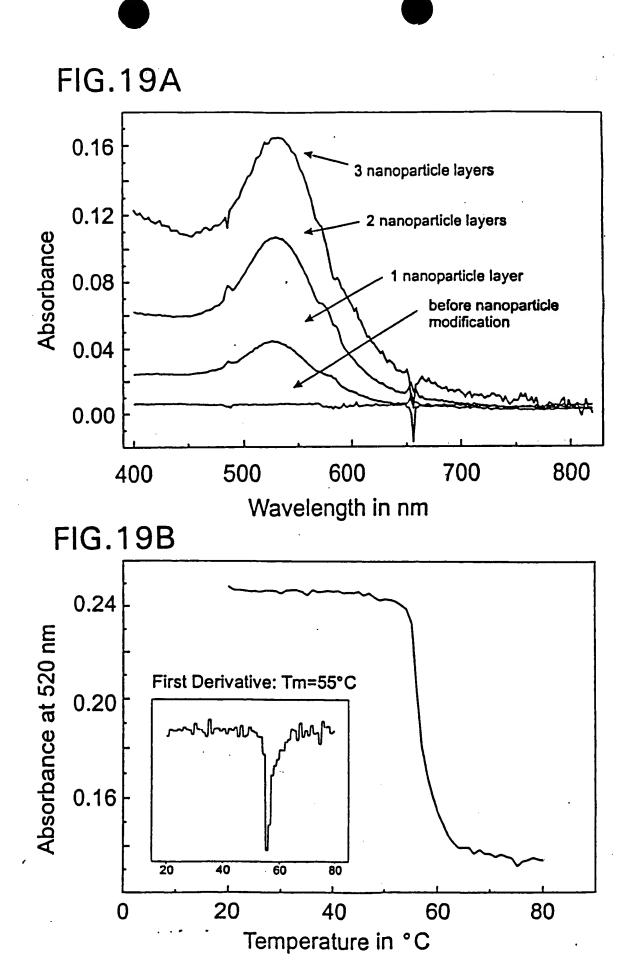
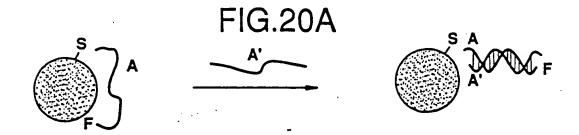
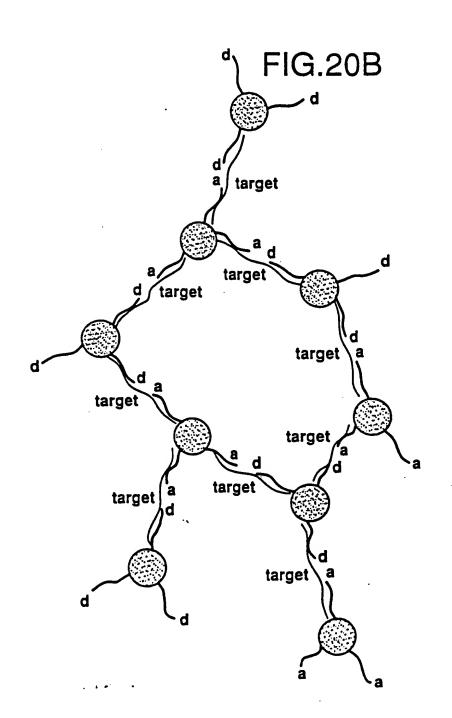


FIG.17E





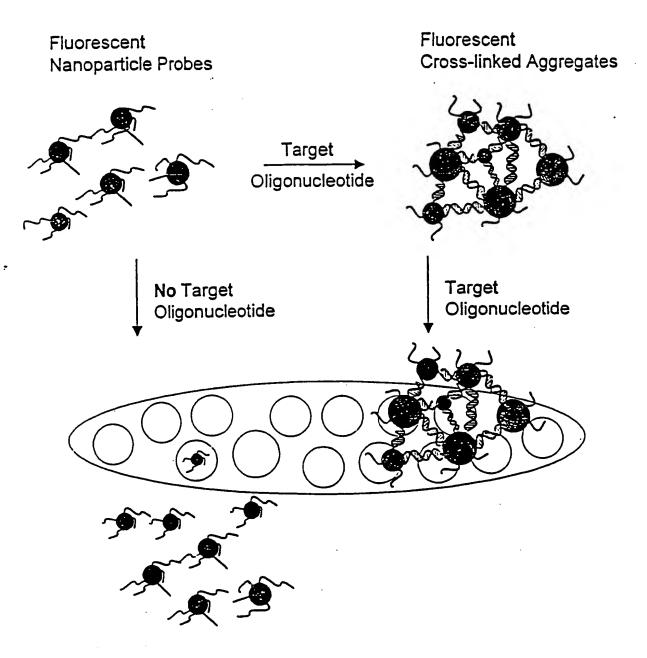




Fluorophore labeled Au/Latex hybrid Oligonucleotide oligonucleotide modified modified Au latex probes nanoparticle probes Target Oligonucleotide Pink/Non-fluorescent White/Fluorescent Red Target No Target Oligonucleotide Oligonucleotide Excess Au probes All Au probes pass pass through through membrane membrane

FIGURE 21

## FIGURE 22



The fluorescent nanoparticle probes pass through the membrane

The fluorescent cross-linked aggregates are retained by the membrane

### **Anthrax PCR Product**

5'G GCG GAT GAG TCA GTA GTT AAG GAG GCT CAT AGA GAA GTA ATT AAT 3'C CGC CTA CTC AGT CAT CAA TTC CTC CGA GTA TCT CTT CAT TAA TTA

TCG TCA ACA GAG GGA TTA TTG TTA AAT ATT GAT AAG GAT ATA AGA AAA AGC AGT TGT CTC CCT AAT AAC AAT TTA TAA CTA TTC CTA TAT TCT TTT

ATA TTA TCC AGG GTT ATA TTG TAG AAA TTG AAG ATA CTG AAG GGC TT 3'
TAT AAT AGG TCC CAA TAT AAC ATC TTT AAC TTC TAT GAC TTC CCG AA 5'

141 mer Anthrex PCR product [SEQ 10 NO:36]

3' CTC CCT AAT AAC AAT

[SEQ ID NO:31]

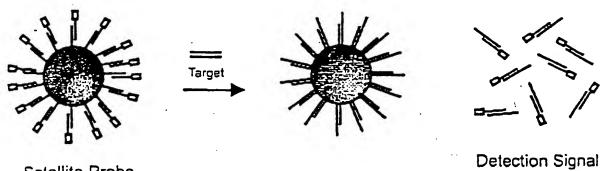
3' TTA TAA CTA TTC CTA ID NO: 38]

Oligonucleotide-Nanoparticle Probes

### Blocker Oligonucleotides

3' C CGC CTA CTC AGT CAT CAA TTC CTC CGA GT [SEQ 1D NO: 39]
3' A TCT CTT CAT TAA TTA AGC AGT TGT [SEQ 1D NO: 40]
3' TAT TCT TTT TAT AAT AGG TCC CAA TAT [SEQ 1D NO: 41]
3' AAC ATC TTT AAC TTC TAT GAC TTC CCG AA [SEQ 1D ND: 42]

FIGURE 23



Satellite Probe

FIGURE 24

## 1. **(**target)

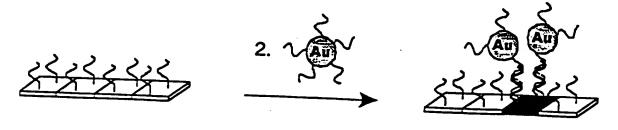


FIGURE 25A

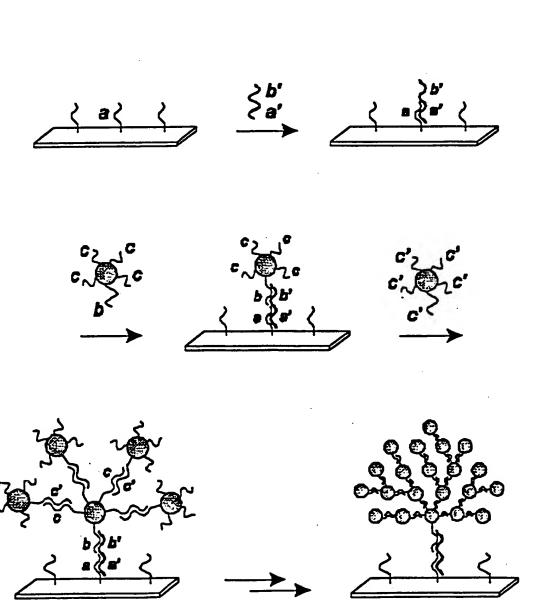


FIGURE 25 B

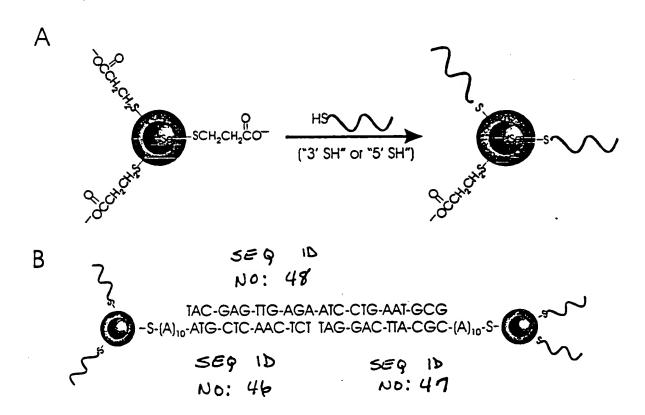
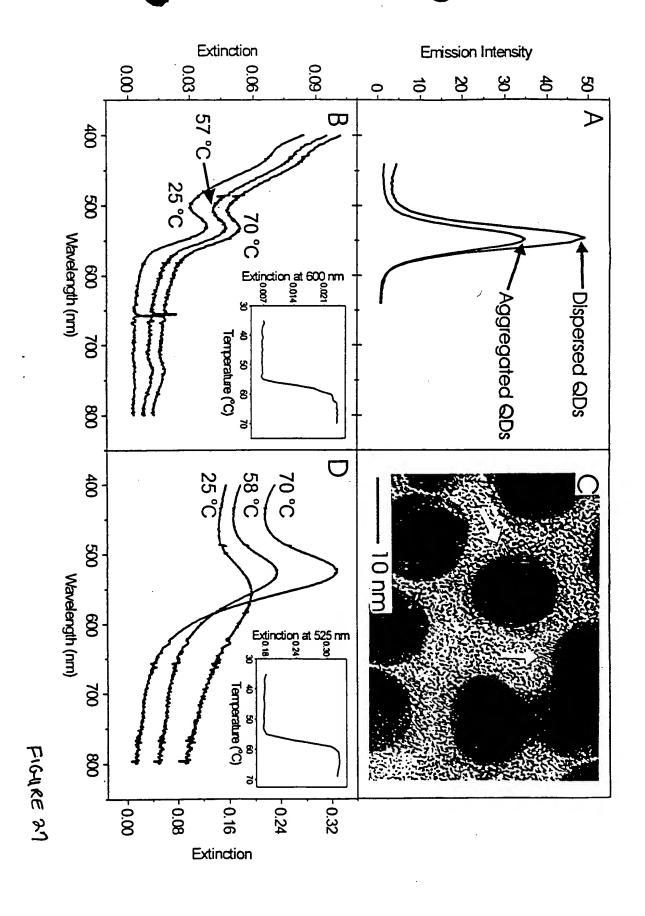
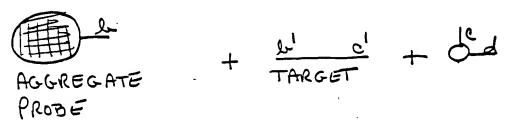
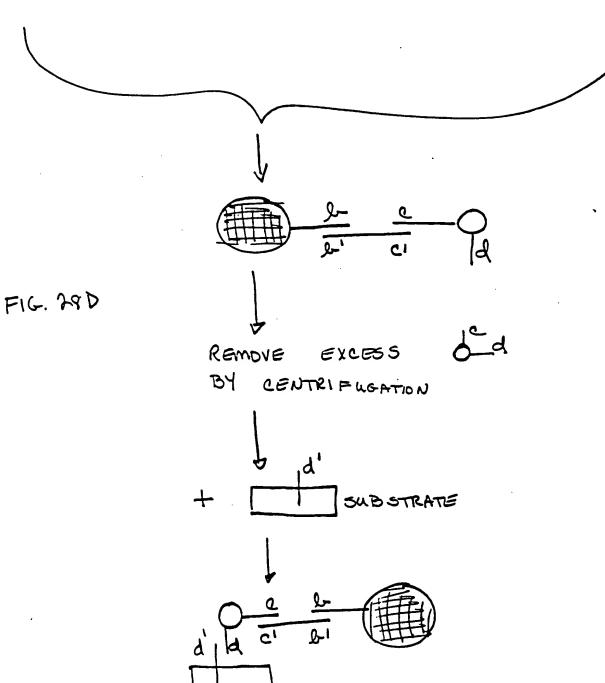


FIGURE 26







$$\frac{a + a' + a' + a'}{a'} \longrightarrow ORE$$

FIGURE Z8A

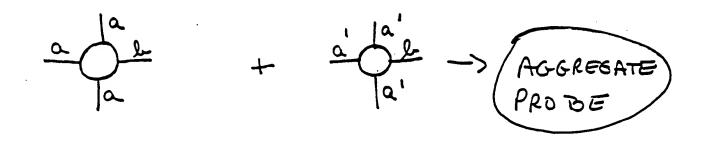
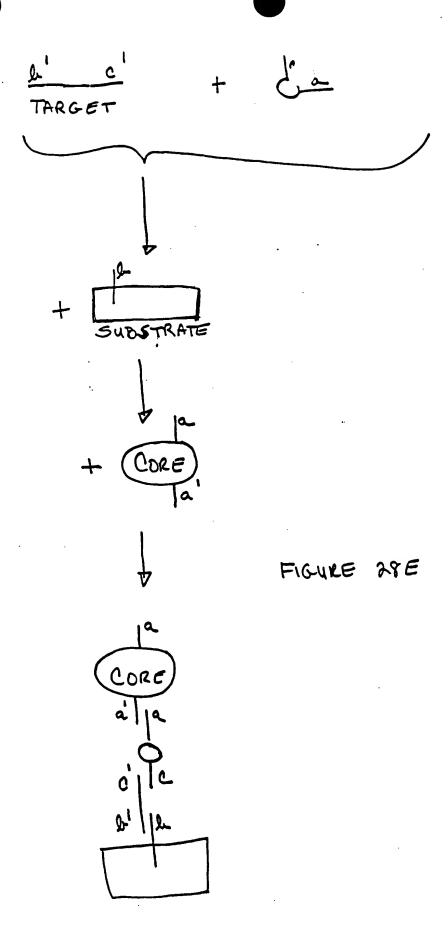


FIGURE A8 B

FIG. 28C



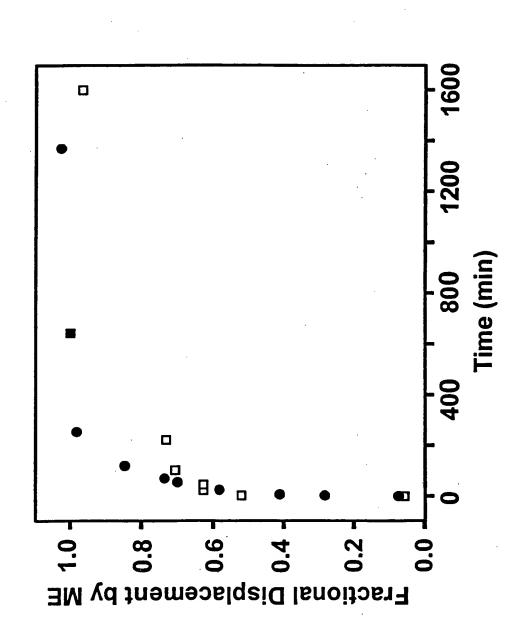


Figure 29

[SEQ ID NO:56]

5' GGA TTA TTG TTA---AAT ATT GAT AAG GAT 3'

CCT ANT AAC AAT TTA TAA CTA TTC CTA

[SEQ ID NO:57] [SEQ ID NO:58]

N = A (complementary), G,C,T (mismatched)

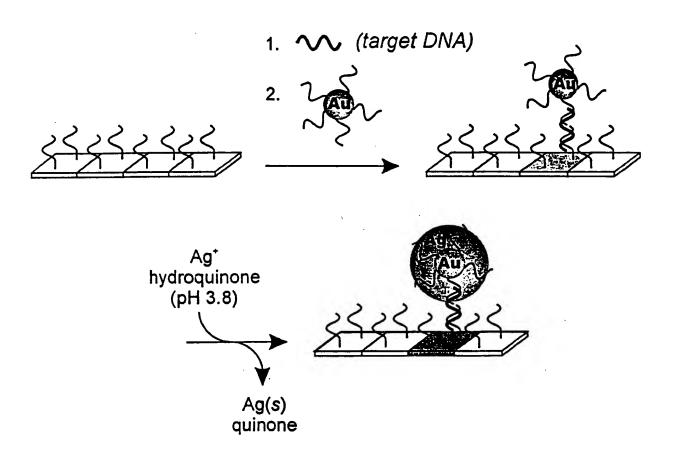


Figure 32

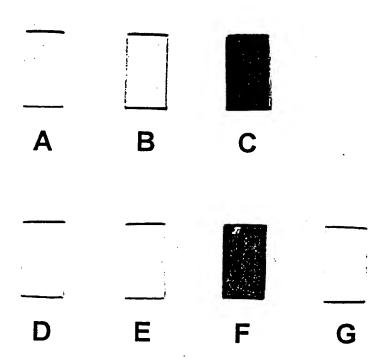


Figure 33

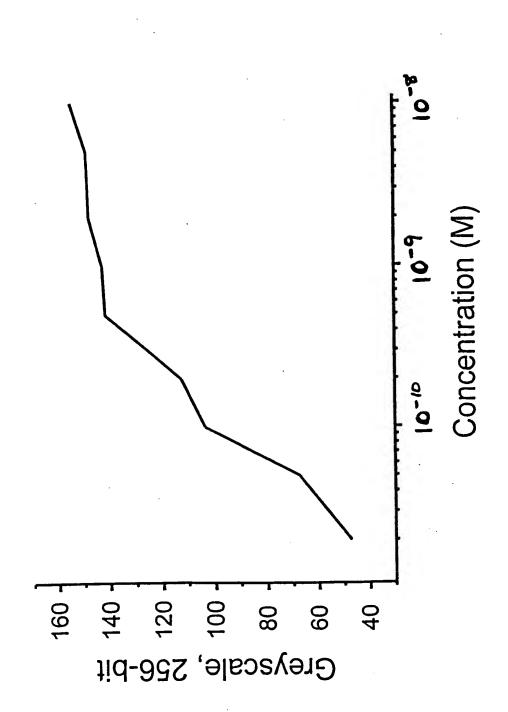


Figure 34

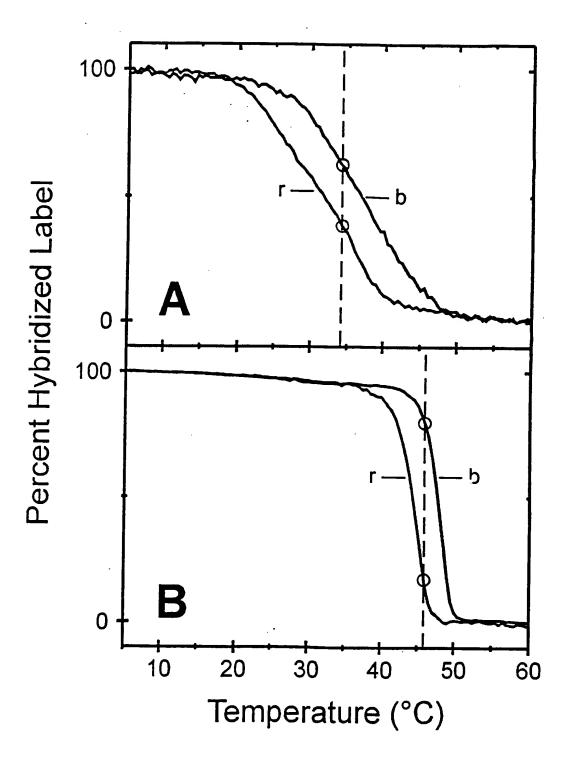


Figure 35

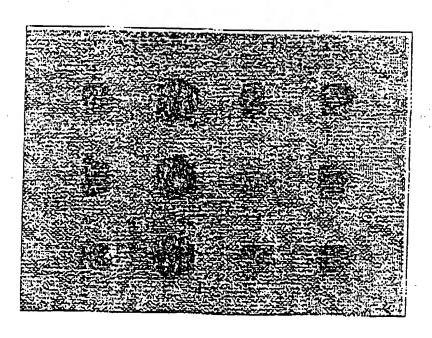
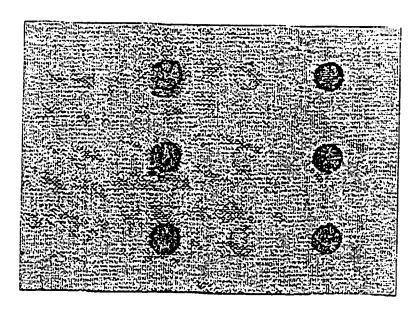


FIG. 368



CATG

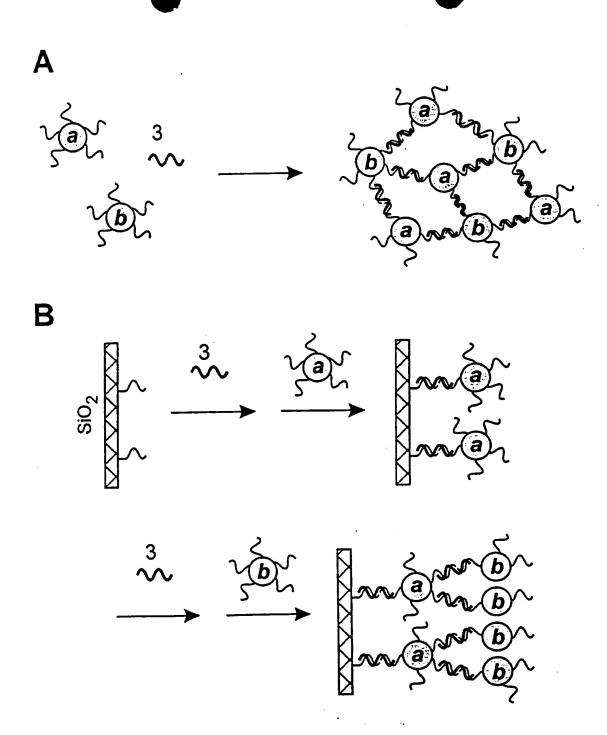


Figure 37

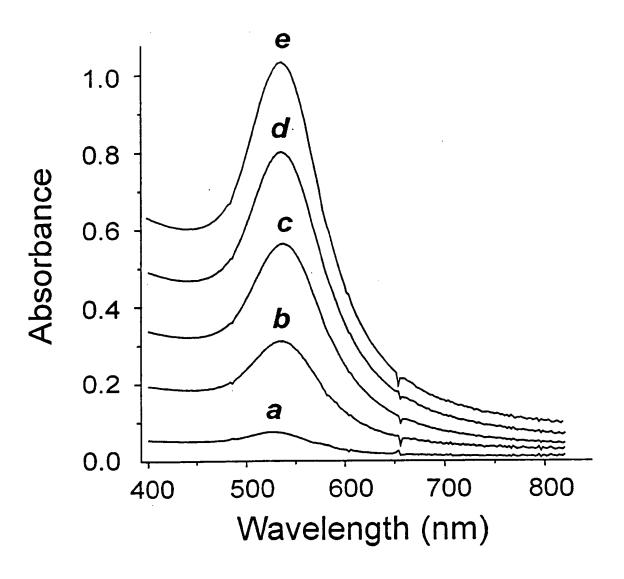


Figure 38A

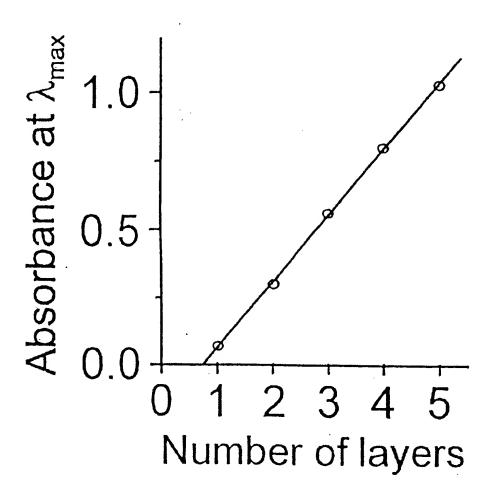


Figure 38B

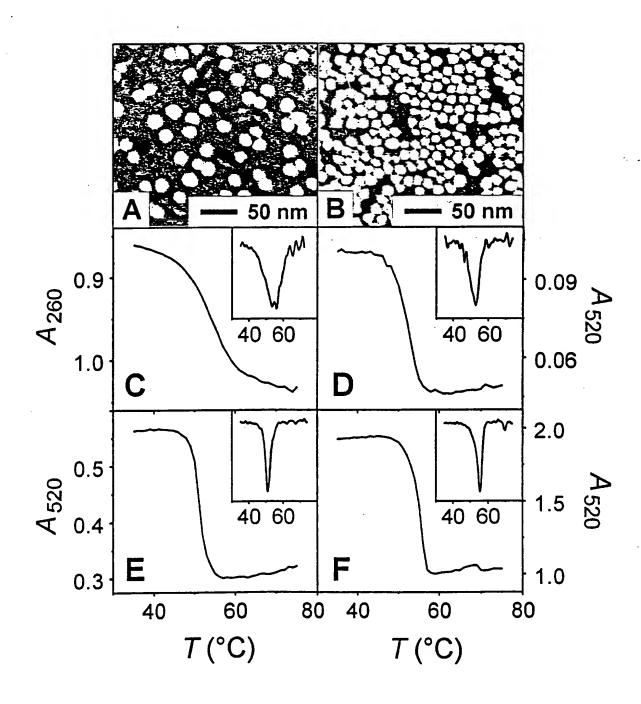
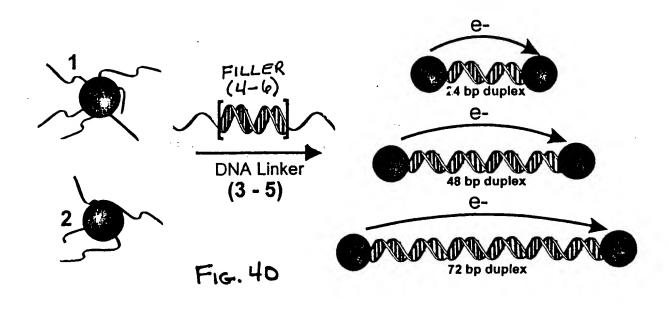


Figure 39



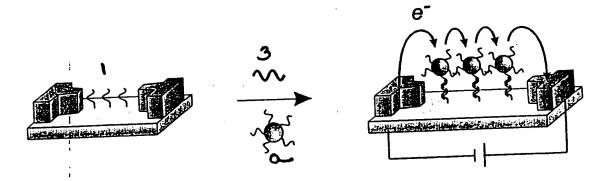


FIG. 41